MICROBIC DECOMPOSITION OF PANTOTHENIC ACID

BY

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MICROBIC DECOMPOSITION OF PANTOTHENIC ACID[1]

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The role of vitamins in the nutrition of microorganisms is well known. In many cases the accessory growth factor functions as a coenzyme, apparently being little affected itself in the process. In direct contrast to this function is the fact that at least some of the vitamins may be attacked and decomposed (either partially or completely) by certain microorganisms. In this case the vitamin serves as a substrate for the particular enzymes of the cell involved, the resulting decomposition yielding energy and materials for possible use by the cell in its various metabolic activities. Relatively little is known regarding the dissimilation of accessory growth factors, but that such can occur is not surprising in view of the wide variety of organic compounds that are subject to the action of microorganisms. Certainly, such information would lead to a better understanding of the metabolism of these vital substances, especially their biosynthesis. The literature on this subject has been reviewed by Koser and Baird (1944).

The present work was undertaken with the thought that information concerning the metabolism of pantothenic acid could be gained if it were possible to find microorganisms capable of decomposing this substance. Such microorganisms were found in soil and air (belonging to the genus *Pseudomonas*), and their action on pantothenic acid is described.

EXPERIMENTAL PROCEDURES

Media and methods. The experimental work resolved itself into two main portions, one being concerned with the decomposition of pantothenic acid in cultures of growing organisms and the other with decomposition of this substance by resting or washed cells. In the former, a simple medium containing pantothenate as the only carbon source was used extensively. This medium, containing the basal salt mixture of Koser and Baird (1944), had the following composition:

$(NH_4)_2HPO_4$	2.0 g
KH_2PO_4	1.5 g
NaCl	5.0 g
$MgSO_4$	0.1 g
Pantothenate	0.1 to 1.0 g*
Distilled H_2O	1,000 ml

* Calcium pantothenate was commonly used in a concentration of 0.01 per cent because of the increasingly heavy precipitate formed with larger amounts. Sodium pantothenate[3] was used in a concentration of 0.1 per cent.

[1] Abstract of thesis presented in partial fulfillment of requirements for the Ph.D. degree.

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[3] Kindly supplied by Dr. A. C. Bratton, Jr., Parke, Davis and Company, Detroit, Michigan.

The pH of the medium was adjusted to 7.5 to 7.8 and autoclaved at 15 pounds' pressure for 10 minutes. This medium will be referred to hereafter as pantothenate broth, the corresponding agar (pantothenate agar) being prepared by adding 1.5 per cent agar to the broth.

Results involving turbidimetric measurements were obtained with a lumetron model 400 G photoelectric colorimeter (wave length 530 mμ).

Manometric experiments were carried out in the conventional manner, using the Warburg technique. Duplicate vessels, maintained at 30 C during experiments, were used in all cases, and to each were added 1.0 ml of M/20 phosphate buffer at pH 7.7, 1.0 ml of washed cells, and 0.5 ml of the substrate (in the side arm), in addition to KOH or H_2SO_4.

Resting cells were obtained by growing the organisms on 0.1 per cent pantothenate plus 0.2 per cent asparagine agar (asparagine increased the yield of cells while not affecting pantothenate utilization) in Kollé bottles for 48 hours at 33 C. The resultant growth was then washed from the agar with M/60 buffer (in most cases a phosphate buffer at pH 7.7), and the suspension was filtered through a thin layer of glass wool and centrifuged. After a second centrifugation in graduated centrifuge tubes, the packed cells were diluted 1:30 with the M/60 buffer, and this suspension was standardized for each experiment in the lumetron colorimeter. The various substrate solutions were carefully prepared and kept in the frozen state until used.

Isolation and identification of organisms. Various samples of soil were assayed for their content of pantothenate-utilizing organisms by means of two enrichment methods. The methods were essentially the same, except in one case pantothenate was added, at intervals, to moist soil, and in the other a small amount of soil was added to pantothenate broth. In both cases, repeated subcultures in pantothenate broth, combined with platings on pantothenate agar, tended to eliminate nonutilizing organisms, at the same time yielding a total of six pure cultures of bacteria that were capable of continued growth in pantothenate broth. These organisms were considered to be pantothenate utilizers (they showed little or no growth in the same medium without pantothenate) and were designated, according to their isolation numbers, as cultures 135, 401, 512, 513, 701, and 702. Three additional bacteria that could utilize pantothenate were obtained by allowing flasks of pantothenate broth to stand open in a laboratory room for several days. These were designated as cultures 2, 8, and 11.

All nine cultures were periodically checked for purity, were transferred weekly in pantothenate broth and onto pantothenate agar slants, and were kept at 33 C at all times. Reserve supplies of active and dried cultures were also maintained.

Attempts to isolate thermophilic pantothenate-utilizing organisms failed.

Partial identification of these organisms was accomplished. All of the cultures were strikingly alike in all of their characteristics: they were gram-negative, aerobic, motile, nonsporeforming, nonpigmented, nongranular, short to medium rods; they utilized glucose, sucrose, maltose, lactose, and mannite without acid

or gas; they were indole-negative and methyl-red- and Voges-Proskauer-negative; they were non-gelatin-liquefying; they reduced litmus milk with an alkaline reaction; they produced H_2S in small amounts; and six cultures reduced nitrate (five to nitrites and one to free nitrogen). Nutrient agar plate colonies were smooth, shiny, and grayish in color. These characteristics tend to place these organisms in the family *Pseudomonadaceae* (see references by Koser and Baird, 1944; Monias, 1928; Bergey *et al.*, 1939). Flagellar stains of cultures 11 and 512, with which most of the experimental work was done, showed that both had polar flagella only (Leifson's BBL flagellar stain). These organisms, therefore, have

TABLE 1

Effect of temperature on growth of Pseudomonas sp.

CULTURE	AGE	BACTERIAL COUNT, PER ML		
		0.01% Ca-pantothenate broth		Salt mixture, no pantothenate
		25 C	33 C	33 C
	hours			
11	0	100,000	80,000	15,000
	24	920,000	3,200,000	20,000
	48	23,000,000	19,600,000	35,000
	72	491,000,000	403,000,000	43,000
	96	500,000,000	480,000,000	50,000
	144	450,000,000	325,000,000	47,000
512	0	207,000	312,000	50,000
	24	1,980,000	950,000	65,000
	48	42,600,000	75,000,000	108,000
	72	520,000,000	606,000,000	121,000
	96	783,000,000	537,000,000	135,000
	144	700,000,000	480,000,000	134,000

been designated as *Pseudomonas* sp., further classification being deemed not only unnecessary but unwise.

RESULTS OF EXPERIMENTS WITH GROWING CELLS

Growth in pantothenate broth. The effect of certain factors on the growth of *Pseudomonas* sp. in pantothenate broth was determined, mainly to arrive at optimum conditions for later work.

From table 1 showing the nutrient agar plate counts obtained with two typical cultures, it is obvious that temperature did not have much influence on growth in calcium pantothenate broth in the range of 25 to 33 C. Also, a prolonged lag phase was exhibited in this medium, rapid multiplication occurring only after 24 hours and continuing up to 72 hours. Little or no growth took place in the basal salt mixture without pantothenate, indicating that pantothenate was in fact being utilized for growth purposes. The low zero hour count in this medium was due to the fact that these tubes were inoculated from a subculture of the same medium to avoid carrying over pantothenate.

The influence of pH on growth was determined by adjusting calcium pantothenate broth to various pH values (5.2, 5.5, 6.0, 6.5, 6.8, 7.2, 7.5, 7.7, and 8.0) with 0.5N HCl and 0.5N NaOH, the final concentration of pantothenate being 0.01 per cent in a total volume of 8.0 ml of medium. The incubation temperature was 33 C, and growth was determined every 24 hours with the lumetron colorimeter. The inoculum per experimental tube, which was the same in other experiments unless otherwise stated, was 0.1 ml of a 72-hour pantothenate broth culture incubated at 33 C. The results showed that all of the cultures responded similarly to variations in pH, the optimum for each being approximately 7.7. A quite rapid fall in turbidity occurred in the less alkaline media

TABLE 2

Effect of pantothenate concentration on growth of Pseudomonas sp.

CULTURE	CONCENTRATION OF PANTOTHENATE	TURBIDITY READINGS	
	μg/ml	3 days	7 days
11	0	98.5	97
	100	70	67
	500	63	61
	1,000	56	52
	5,000	58	54
	10,000	60	54
	100,000	73	63
512	0	96	94
	100	75	73
	500	67	65
	1,000	60	59
	5,000	63	60
	10,000	68	63
	100,000	79	66

(no growth took place below pH 5.5), and a slight decrease was observed also at pH 8.0. After 168 hours the pH of each medium was within 0.1 to 0.2 of a point of uninoculated controls, indicating that this factor (i.e., a change in pH during growth) would have no effect on growth.

To determine the optimum concentration of pantothenate for growth, sodium pantothenate in amounts of 0, 100, 500, 1,000, 5,000, 10,000, and 100,000 μg per ml was added to the basal salt mixture, and turbidity readings were made after 3 days' incubation at 33 C and again after 7 days. It was found that 1,000 μg per ml was optimum for both of the cultures studied, although 5,000 μg per ml was practically as effective (table 2). Larger amounts of pantothenate, especially 100,000 μg per ml, were definitely inhibitory to growth, although not completely so.

Another experiment showed that growth in pantothenate broth was the same whether the pantothenate was added to the medium before autoclaving or

whether a filtered solution of pantothenate was added aseptically to the autoclaved basal salt mixture. The addition of certain inorganic salts ($ZnCl_2$, $CaCl_2$, $MnCl_2$, and $FeSO_4$) did not improve nor impair growth in pantothenate broth.

Two analogues of pantothenic acid, pantoyltaurine[4] and *dl*-N-pantoyl-*n*-butylamine,[5] were studied for their effect on the growth of *Pseudomonas* sp. in pantothenate broth. It was found that the former could support growth when used in a concentration of 10,000 μg per ml in the absence of pantothenate but not to the same extent as did the same concentration of pantothenate in the absence of pantoyltaurine. When both substances were present in the same medium, growth was considerably less than that in a medium which contained only pantothenate, unless the concentration of pantothenate was equal to or greater than the concentration of pantoyltaurine. Use of the same procedure with *dl*-N-pantoyl-*n*-butylamine, revealed that this substance (in a concentration of 10,000 μg per ml) did not support growth, nor did added pantothenate cause growth unless an excess was present. With the reverse procedure (i.e., varying the concentration of the analogue and holding the pantothenate level constant) essentially the same relationship between these substances was noted. It is obvious that these results demonstrate a competitive type of inhibition.

Growth in modified pantothenate broth. To determine whether the components of pantothenic acid could be utilized for the growth of *Pseudomonas* sp., various media were made up as follows: β-alanine broth (40 μg and 300 μg per ml); α-hydroxy-β,β-dimethyl-γ-butyrolactone[6] broth (60 μg and 300 μg per ml); α,γ-dihydroxy-β,β-dimethyl-butyric acid (pantoic acid) broth (60 μg and 300 μg per ml); and various combinations of these media. Pantoic acid was prepared from the lactone according to the method of Sarett and Cheldelin (1945). These media were prepared as usual except that pantothenate was replaced by the compound or compounds under study in the concentrations indicated. All nine cultures were used in these tests, and the results were obtained by visual observations of turbidity. It was found that both β-alanine and pantoic acid supported growth, the amount of growth increasing in each case as the concentration of the substrate was increased. When combined, these substances gave growth approximately equal to that in control tubes of pantothenate broth. In no instance did the lactone support growth of any of the cultures, nor did it increase the effectiveness of β-alanine broth. It seems obvious that these organisms were unable to break the lactone ring, but when this structure is ruptured (as in pantoic acid) the compound could be utilized.

In another experiment, it was found that growth in pantothenate broth was not affected by removing $(NH_4)_2HPO_4$ from the basal salt mixture. By also excluding atmospheric nitrogen, it was apparent that these organisms could

[4] Kindly supplied by Dr. F. A. Robinson, The Glaxo Laboratories, Ltd., Greensford, Middlesex, England.

[5] Kindly supplied by Dr. William Shive, University of Texas, Austin, Texas.

[6] Kindly supplied by Dr. D. F. Robertson, Merck and Company, Inc., Rahway, New Jersey.

deaminate β-alanine as a source of nitrogen. The salt was retained, however, as an extra nitrogen supply.

The effect of added nutrients upon the growth of *Pseudomonas* sp. in pantothenate broth was studied in order to try to improve the yield of bacteria for future work involving washed cells. Difco asparagine (0.2, 0.4, and 0.6 per cent) and smaco acid-hydrolyzed casein (0.5 per cent) each greatly improved growth of five of the cultures tested, as determined by visual turbidity. As was shown next, however, casein hydrolyzate "spared" the pantothenate, to a large extent, from being acted upon, whereas asparagine did not.

Destruction of pantothenate during bacterial growth. In order to prove that pantothenate was actually being decomposed during growth of these *Pseudomonas* cultures and to determine the rate of this decomposition, microbiological assays for pantothenate were carried out by the method of Skeggs and Wright (1944). With 0.1 per cent sodium pantothenate broth, it was found that all nine of the cultures had destroyed 100 per cent of the substrate within a growth period of 72 hours at 33 C. After 24 hours, cultures 11 and 512 (the only ones tested) had destroyed 20 per cent of the pantothenate.

To determine the effect of added nutrients upon the destruction of pantothenate, 0.2 per cent asparagine and 0.5 per cent casein hydrolyzate were added, respectively, to pantothenate broth. The media were inoculated with cultures 11 and 512, and the contents of each were assayed for their pantothenate concentration after 24 and 72 hours' incubation at 33 C. The results with asparagine were the same as those obtained in its absence, but with casein hydrolyzate no breakdown was detected with either culture at 24 hours, and at 72 hours less than 50 per cent decomposition of pantothenate had occurred. Therefore, the use of casein hydrolyzate was discontinued, but asparagine was later incorporated in pantothenate agar for the production of washed cells.

Survey of other microorganisms for pantothenate utilization. It was of interest to determine whether various representative stock cultures of bacteria or fungi possessed any native ability to decompose pantothenic acid. The methods used seemingly afforded the microorganisms optimum conditions for attacking pantothenate, but in all cases the results were negative. Therefore, for brevity, the details of this work will not be described. It is reasonable to assume, however, that in nature many of these same microorganisms may play an active role in decomposing pantothenate, having lost this function on repeated transfer in the laboratory.

RESULTS OF EXPERIMENTS WITH RESTING CELLS

Deamination and Thunberg studies. An investigation into the deamination of pantothenate was performed by adding 1.0 ml of washed cells (preparation previously explained), 1.0 ml of M/20 phosphate buffer at pH 7.5, and 3.0 ml of M/50 substrate to duplicate test tubes and determining ammonia production with Nessler's reagent after incubation periods of 2, 6, and 24 hours at 33 C. It was found that pantothenate was deaminated and that the reaction progressively increased from 2 to 24 hours, meaning that the β-alanine portion

of pantothenic acid was actually the substance being acted upon. A similar experiment with β-alanine did, in fact, give the same results as were obtained with pantothenate. Also, similar results were obtained with *dl*-alanine, this substance being included to determine the specificity of the deaminase. These findings are in agreement with other reports (Stephenson, 1939) that pseudomonae can deaminate various amino acids.

Preliminary to carrying out manometric experiments, it was of interest to determine whether a representative culture, culture 11, could reduce methylene blue with β-alanine, pantoic acid, pantoyl-lactone, and pantothenate as substrates. The usual Thunberg technique was used, in which 1.0 ml of washed

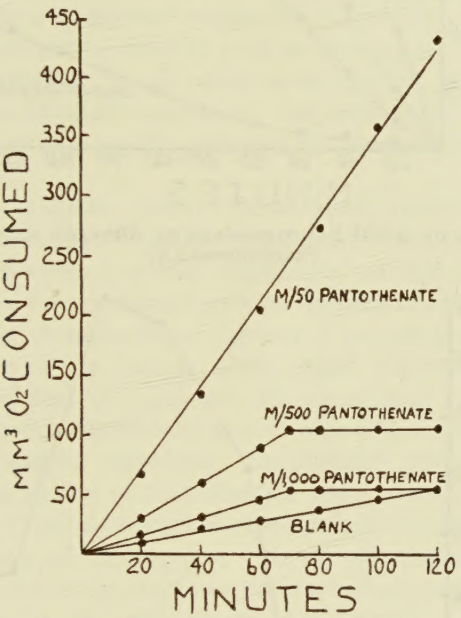

FIG. 1. THE OXIDATION OF PANTOTHENATE BY PSEUDOMONAS SP., CULTURE 11

cells, 1.0 ml of M/20 phosphate buffer at pH 7.5, 0.5 ml of 1:10,000 methylene blue, and 0.5 ml of M/50 substrate were placed in duplicate Thunberg tubes, the tubes evacuated, the cells tipped in from the side arm at the zero time, and the tubes incubated at 33 C and observed visually at 5-minute intervals for decolorization. It was found that the methylene blue was completely decolorized in 65 minutes with pantothenate as the substrate, in 80 minutes with β-alanine, and in 75 minutes with pantoic acid. Tubes containing the lactone remained blue for a much longer period of time, decolorizing at the same rate as the controls.

Warburg studies; oxidation of pantothenate. From the foregoing experiment, it was expected that pantothenate would be readily oxidized in the Warburg apparatus. This was found to be true, and the oxidation of various concentrations of pantothenate by culture 11 is shown in figure 1. It is apparent that

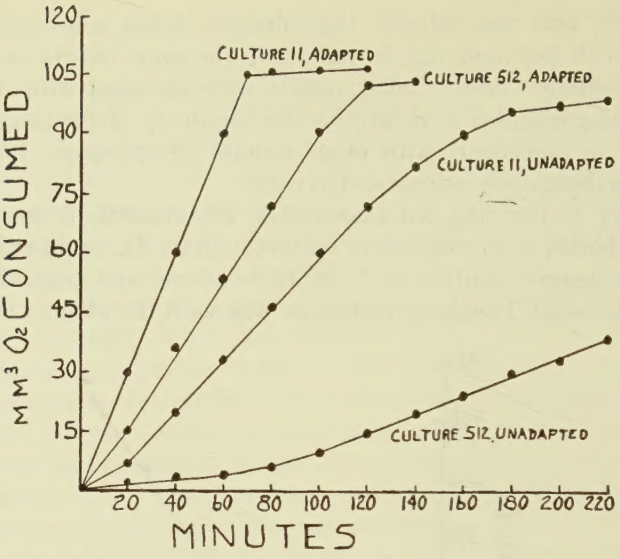

FIG. 2. THE OXIDATION OF M/500 PANTOTHENATE BY ADAPTED AND UNADAPTED CELLS OF PSEUDOMONAS SP.

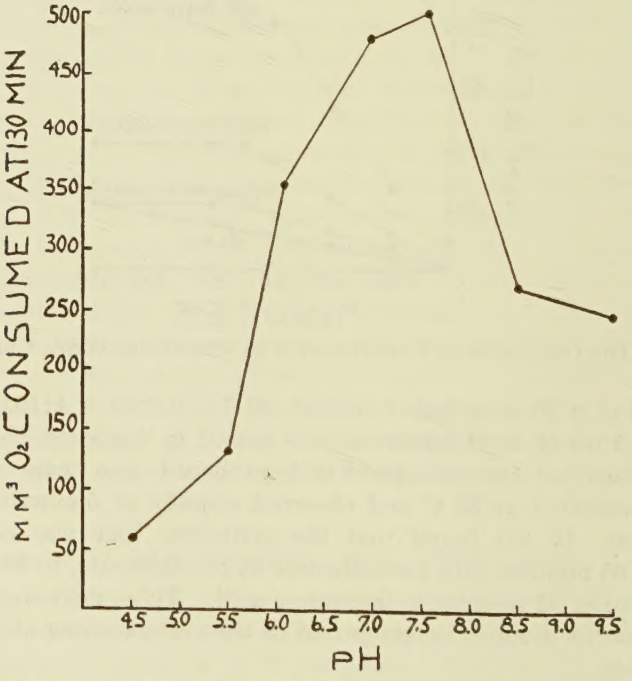

FIG. 3. THE EFFECT OF pH ON THE OXIDATION OF M/50 PANTOTHENATE BY PSEUDOMONAS SP., CULTURE 11

the reaction with M/500 and M/1,000 substrates reaches completion in approximately 70 minutes, at which time about 50 per cent of the total oxygen (theo-

retical) needed for complete combustion has been used. Although carbon dioxide curves were not established, it was found that after 70 minutes a total of 90 mm^3 of this gas had been evolved from M/500 pantothenate, this being equivalent to 4 molecules of carbon dioxide. Since the oxygen consumption of this substrate was equal to 5 molecules, the respiratory quotient (R.Q.) at 50 per cent oxidation was 0.80 as against a theoretical R.Q. of 0.90 for complete combustion.

Cultures 512 and 702 required 120 and 200 minutes, respectively, to achieve 50 per cent oxidation of M/500 pantothenate, but it is noteworthy that they, too, eventually completed the reaction at the same end point as did culture 11. Of the three, only culture 702 exhibited any significant lag period at the beginning of the reaction. The endogenous respiration, especially of culture 11, was rather high, but attempts to lower it, such as aerating the cells for 1 hour prior to use, were not successful. In all cases corrections for the blank were made since it has not been definitely established that endogenous respiration is suppressed in the presence of a readily utilizable substrate, although such may be the case.

The adaptive nature (Dubos, 1940) of the enzymes involved was shown by the fact that 48-hour nutrient agar cultures (unadapted) were much less active than were adapted cultures which had been maintained on pantothenate agar (figure 2). Three transfers of the unadapted cultures on pantothenate-asparagine agar, however, fully adapted them for the utilization of pantothenate.

A study into some of the factors which might influence the oxidation of pantothenate revealed that the optimum pH was around 7.7 (figure 3). M/50 substrate was used in this experiment so that any differences in activity at the various pH levels would be magnified. An intensive study into the effect of temperature on the oxidation of pantothenate was not made, but it was found that an increase to 38 C (all experiments were conducted at 30 C) neither affected the rate nor the degree of the reaction. It was also found that the enzymes involved in the oxidation were stable for at least 10 days when stored (in the form of packed cells) in the refrigerator (not frozen). Thus, washed cells were usually prepared a day before use and stored in the packed state overnight in the refrigerator. Also, it was revealed that physiologically young cells (48 hours) were much more active than 96-hour (or older) cells. This was expected but, unfortunately, owing to insufficient yield, 24-hour cells could not be used.

Various attempts to explain the incomplete oxidation of pantothenate were unsuccessful and, without going into the details of this work, it may be said that the only logical explanation for this phenomenon was that a reaction of oxidative assimilation was occurring, the nature of which will be briefly discussed later.

A survey of four nutrient agar stock cultures of bacteria, namely *Escherichia coli-communior*, *Proteus vulgaris*, *Acetobacter suboxydans*, *and Pseudomonas aeruginosa* showed that the first three had absolutely no activity on M/50 pantothenate, and *P. aeruginosa* had only a very minimal activity, which was not increased by three transfers on pantothenate-asparagine agar.

Experiments with pantothenate analogues showed that M/50 *dl*-N-pantoyl-*n*-

butylamine was not oxidized and, moreover, did not interfere with the oxidation of M/500 pantothenate when both were present in the same vessel. Apparently this substance inhibits growth by some mechanism other than an interference with pantothenate utilization. Pantoyltaurine was oxidized to some extent but the taurine component was not, indicating that taurine may interfere with the metabolism of pantoic acid as well as that of β-alanine, since pantoyltaurine was not oxidized to the same extent as was pantoic acid alone.

Oxidation of β-alanine and pantoic acid. Figure 4 shows the oxidation of M/50 and M/500 β-alanine and M/500 pantoic acid by culture 11. The reaction with both components was completed in 100 minutes and, by appropriate calculations, it was determined that M/500 β-alanine was oxidized to two-thirds of completion and M/500 pantoic acid to approximately 43 per cent of completion, 2 and 3 molecules of oxygen having been consumed, respectively. The decided

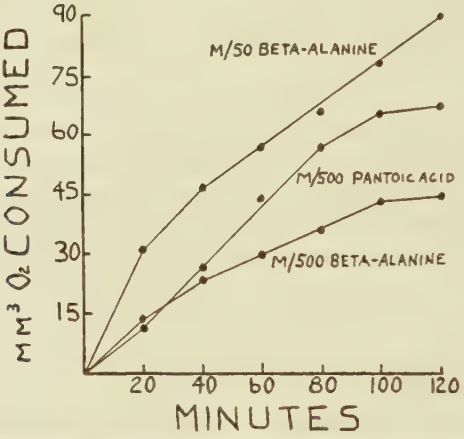

FIG. 4. THE OXIDATION OF β-ALANINE AND PANTOIC ACID BY PSEUDOMONAS SP., CULTURE 11

break in the oxidation rate of β-alanine after 20 minutes is impossible to explain (M/1,000 substrate, not shown— presented the same type of curve). After 100 minutes, 2 molecules of carbon dioxide had been evolved from M/500 β-alanine and a like number from pantoic acid, giving respective R.Q. values of 1.0 and 0.67 as against theoretical R.Q. values of 1.0 and 0.86, respectively, for complete oxidation. Results similar to those obtained for β-alanine were reported by Webster and Bernheim (1936) for *dl*-alanine, using *Pseudomonas aeruginosa*. When both β-alanine and pantoic acid were present in the same vessel, the oxidative curve was similar to that obtained with an equivalent amount of pantothenate. The lactone moiety of pantothenic acid was not oxidized by culture 11, using M/50 and M/500 concentrations.

Experiments with cell poisons. As noted earlier, the incomplete oxidation of pantothenate suggested a type of reaction described many times before (Clifton, 1946) and called oxidative assimilation. Briefly, the reaction is one in which the substrate, under normal conditions, is not completely oxidized, but

instead a substance, or substances, with the empirical formula of a carbohydrate is formed. This substance, then, is assimilated by the cells, preventing any further degradation. The addition of an appropriate enzyme inhibitor, however, prevents the processes of assimilation from continuing, allowing dissimilation to proceed.

With KCN as the inhibitor of assimilation in this case, it was found that the oxidation of M/500 pantothenate was greatly increased in the presence of certain

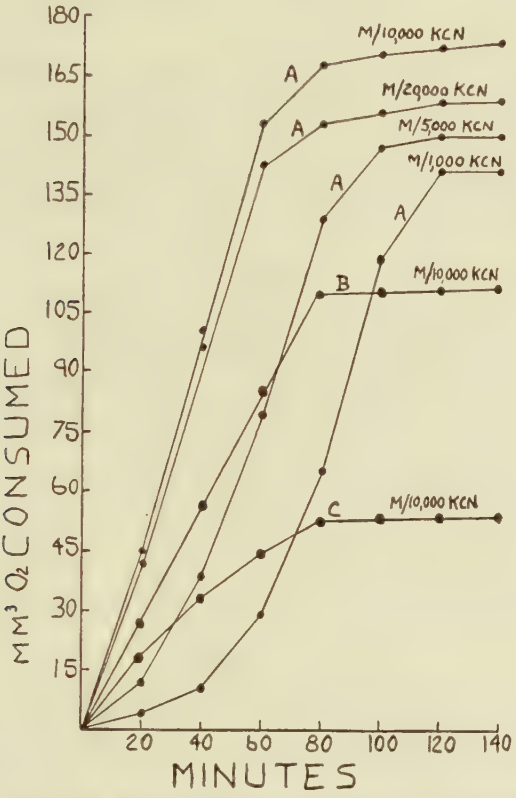

FIG. 5. THE EFFECT OF KCN ON THE OXIDATION OF M/500 PANTOTHENATE (A), M/500 PANTOIC ACID (B) AND M/500 β-ALANINE (C) BY PSEUDOMONAS SP., CULTURE 11

concentrations of cyanide (figure 5), the reaction proceeding to 78 per cent of completion with M/10,000 KCN. Similar results were obtained with β-alanine and pantoic acid, the former going to approximately 80 per cent of completion and the latter to approximately 71 per cent of completion in the presence of M/10,000 KCN (figure 5). In each case carbon dioxide evolution also increased, the R.Q. values more nearly approaching those for complete dissimilation of each respective substrate (table 3).

These results leave no doubts that the normal oxidation of pantothenate (and its components) is one of oxidative assimilation. Therefore, the normal

oxidation of these substances may be represented by the following balanced equations:

$$C_3H_7O_2N + 2O_2 = 2CO_2 + H_2O + NH_3 + (CH_2O) \quad (1)$$
β-alanine

$$C_6H_{12}O_4 + 3O_2 = 2CO_2 + 2H_2O + 4(CH_2O) \quad (2)$$
pantoic acid

$$C_9H_{17}O_5N + 5O_2 + H_2O = 4CO_2 + 3H_2O + NH_3 + 5(CH_2O) \quad (1 + 2)$$
pantothenic acid

Attempts to show a 100 per cent decomposition of pantothenate with other enzyme inhibitors were unsuccessful. Monoiodoacetic acid (M/5,000 and M/50,000) greatly inhibited the oxidation of M/500 pantothenate, whereas sodium azide gave results very similar to those obtained with KCN, but in different concentrations. Endogenous respiration was not significantly affected by any of the inhibitors.

TABLE 3

Comparative results of oxygen consumption and carbon dioxide evolution with and without potassium cyanide (M/10,000)

SUBSTRATE	OXYGEN				CARBON DIOXIDE				R.Q.	
	No KCN		With KCN		No KCN		With KCN		No KCN	With KCN
	mm³	mol.	mm³	mol.	mm³	mol.	mm³	mol.		
M/500 Pantothenate...	110	5	175	7.8	90	4	146	6.5	0.80	0.83
M/500 β-Alanine.....	45	2	54	2.4	45	2	54	2.4	1.0	1.0
M/500 Pantoic acid....	66	3	112	5.0	45	2	88	3.9	0.67	0.78

DISCUSSION

The results of this study again tend to emphasize the microbic decomposition of vitamins in contrast to their usual role as accessory growth factors. It is striking that most of the studies of this nature have been done with pseudomonae and of significance that in each case the organisms were isolated directly from soil or other natural habitats, undoubtedly involving a process of natural adaptation. What importance these studies have in relation to the decomposition of vitamins in the human intestinal tract is unknown.

In the present study it was found that the lactone moiety of pantothenic acid could neither serve as a growth substrate nor as an oxidizable substrate for *Pseudomonas* sp., whereas its hydrolyzed counterpart, pantoic acid, was readily utilized by both growing and resting cells. This is interesting in view of the fact that Stansly and Schlosser (1945) reported that pantoic acid is more readily utilized than is the lactone for the synthesis of pantothenic acid by *Escherichia coli*. They stated that pantoic acid is the probable precursor in the biological synthesis of pantothenic acid, rather than pantolactone.

Although the evidence points undeniably to a process of oxidative assimilation in the decomposition of pantothenate, it is true that a carbohydrate has not been actually demonstrated. Giesberger (1936), in similar experiments, did show an increase in the volutin content of *Spirillum serpens*, but most investigators have not studied this particular problem in detail. Suffice it to say that, knowing all of the facts, no other logical explanation of the phenomenon is possible.

It is realized that actively proliferating cells may not act in the same manner on pantothenate as do resting cells, but no attempt was made here to determine this relationship. Whelton and Doudorff (1945), however, did show that both types of cells of *Pseudomonas saccharophila* assimilated some substrates in essentially the same manner and other substrates in quite a different manner.

From the results obtained here, it appears that the carbohydrate substance produced during the oxidation of pantothenate is formed with great economy by the bacterial cells, approximately 55 per cent of the carbon of the substrate being assimilated. In similar studies, Barker (1936) found that the alga, *Prototheca zopfi*, converts from 50 per cent to more than 80 per cent of the carbon of various substrates into a carbohydrate material. This undoubtedly explains the ability of some of these microorganisms to survive and multiply in simple media.

The manner in which bacterial cells form this carbohydrate from a substance such as pantothenic acid is not clearly evident, although Clifton and Logan (1939) have postulated a theory, from known facts, for the formation of carbohydrate from various substrates by cells of *Escherichia coli*. Presumably, the process represents more than a mere reduction of carbon dioxide. Equally intriguing is the manner in which a poison like KCN selectively blocks the assimilatory process.

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SUMMARY

Bacteria of the genus *Pseudomonas* have been isolated from soil and air that could utilize pantothenate as a growth substrate in a medium containing only pantothenate and inorganic salts. β-Alanine and pantoic acid also were utilized when substituted for pantothenate, but pantoyl-lactone did not support growth.

During their growth, these bacteria decomposed 20 per cent of the pantothenate in 0.1 per cent pantothenate broth within 24 hours and 100 per cent of the substrate within 72 hours.

Pantoyltaurine and *dl*-N-pantoyl-*n*-butylamine, analogues of pantothenic acid, exhibited a competitive type of inhibition with pantothenate but only the former was able to support growth when substituted for pantothenate, and then to a lesser extent.

Manometric studies showed that pantothenate, β-alanine, and pantoic acid were oxidized, respectively, to 50 per cent, 67 per cent, and 43 per cent of completion by a process of oxidative assimilation. Pantoyl-lactone was not oxidized.

Potassium cyanide and sodium azide, in critical concentrations, caused the

oxidation of pantothenate, β-alanine, and pantoic acid more nearly to reach completion, presumably by inhibiting the processes of assimilation.

dl-N-Pantoyl-*n*-butylamine was not oxidized and pantoyltaurine was only slightly oxidized in the Warburg apparatus.

Various stock cultures of bacteria and fungi were not able to utilize pantothenate as a carbon source, nor were they able to oxidize this substance.

The significance of these findings is discussed.

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